EMBO reports

review

Genetic manipulation of insulin signaling, action and secretion in mice

Insights into glucose homeostasis and pathogenesis of type 2 diabetes

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Received November 20, 2001; revised February 19, 2002; accepted February 26, 2002

Non-insulin-dependent diabetes mellitus (NIDDM) is a complex heterogeneous polygenic disease characterized mainly by insulin resistance and pancreatic β-cell dysfunction. In recent years, several genetically engineered mouse models have been developed for the study of the pathophysiological consequences of defined alterations in a single gene or in a set of candidate diabetogenes. These represent new tools that are providing invaluable insights into NIDDM pathogenesis. In this review, we highlight the lessons emerging from the study of some of the transgenic or knockout mice in which the expression of key actors in insulin signaling, action or secretion has been manipulated. In addition to contributing to our knowledge of the specific roles of individual genes in the control of glucose homeostasis, these studies have made it possible to address several crucial issues in NIDDM that have remained controversial or unanswered for a number of years.

Introduction

Insulin is a peptide hormone secreted by pancreatic β-cells and plays a vital role in the control of glucose homeostasis by regulating carbohydrate, lipid and protein metabolism. Although the insulin receptor (IR) is widely distributed throughout the body, the three major target tissues for its metabolic actions are muscle, liver and fat. The IR belongs to the family of membrane receptors with tyrosine kinase activity (Ullrich and Schlessinger, 1990), and the mechanisms of insulin signaling are now understood in great detail (White and Kahn, 1994). The activated receptor phosphorylates intracellular 'docking' proteins such as the insulin receptor substrates (IRSs), Src homology and collagen-like protein (Shc) or Grb2-associated binder 1 (Gab-1), which subsequently recruit various intracellular effector proteins. This, in turn, results in the activation of signaling pathways such as the phosphatidylinositol 3-kinase (PI 3-kinase) or the mitogen-activated protein kinase (MAP kinase) pathways, leading to a variety of biological effects in target cells (Figure 1).

A collapse in glucose homeostasis results in diabetes mellitus, which is characterized by high blood glucose levels (hyperglycemia), and, if untreated, leads to glucose release in the urine (glycosuria). Chronic hyperglycemia is considered to be the main factor responsible for the development of diabetes-associated retinal, renal, neurological and vascular complications. There are two forms of diabetes, and the focus of this review, non-insulin-dependent diabetes mellitus (NIDDM), or type 2 diabetes, afflicts >110 million people worldwide. Apart from some monogenic forms, NIDDM is a heterogeneous, polygenic disease with a complex inheritance pattern. Environmental factors such as lack of exercise or diet also contribute to the development of the disease. NIDDM is characterized by two major pathophysiological lesions: insulin resistance and β-cell dysfunction (De Meyts, 1993; Kahn, 1994; De Fronzo, 1997). Insulin resistance reduces the ability of insulin to stimulate glucose uptake and utilization in skeletal muscle and adipose tissue and to suppress hepatic glucose production (Figure 2). This, in turn, is initally associated with increased insulin secretion (hyperinsulinemia) in order to overcome insulin resistance. Finally, NIDDM develops when the β-cells fail to secrete adequate amounts of insulin. There have been raging debates about the relative order in which these defects appear and about their relative contributions to the development of the disease. The culprit gene(s), as well as the mechanism(s) that lead to these defects, are still not completely understood. It has also been difficult to evaluate the relative importance of insulin resistance in different target tissues in NIDDM pathogenesis.

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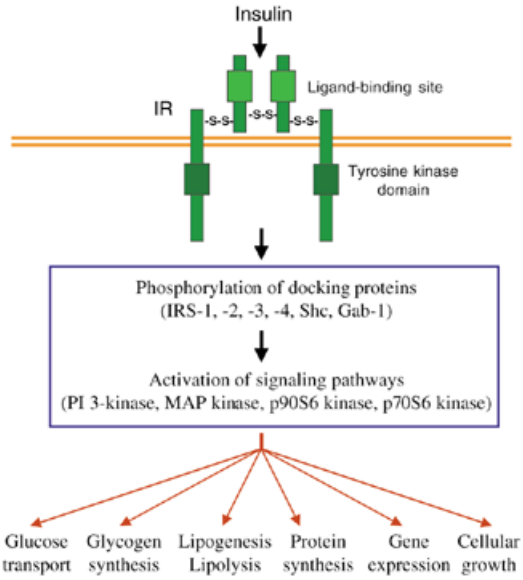

Fig. 1. Insulin signaling through IR leads to pleiotropic effects in target cells.

Genetically engineered mice with clearly defined genetic alterations represent powerful tools for understanding polygenic diseases such as NIDDM. In recent years, the expression of a number of genes encoding key players in insulin signaling, action or secretion has been manipulated in the mouse using transgenic or gene targeting approaches. This review focuses on a number of mouse models (Table I) that have provided novel insights into the mechanisms behind glucose homeostasis and have allowed several key issues pertaining to NIDDM pathogenesis to be addressed. Mice carrying monogenic defects have also been used to generate the first polygenic models of NIDDM.

Mouse models with defective insulin signaling

The crucial role of IR in insulin action was confirmed by targeted disruption of the IR gene. IR-deficient mouse pups developed a severe form of diabetes, with massive fatty acid infiltration in the liver (steatosis) and increased production of ketone bodies and CO_2 (ketoacidosis) (Accili *et al.*, 1996; Joshi *et al.*, 1996). These mutants presented hyperglycemia, hyperinsulinemia, increased serum triglycerides and reduced hepatic glycogen, and they died within 1 week of birth. An unexpected observation was that $IR^{-/-}$ pups were normal at birth, whereas several human patients with different mutations in the IR gene, including a homozygous null mutation, presented severe intrauterine growth retardation. This cautions against extrapolating findings in mice to pathology in man. Heterozygous $IR^{+/-}$ mice did not present any metabolic abnormalities and their ability to normalize blood glucose levels after a glucose challenge (glucose tolerance) was comparable to that of wild-type controls. This suggests that a decrease in IR number does not lead to insulin resistance, challenging the classical concept that IR down-regulation alone resulting from chronically elevated insulin levels could generate severe insulin resistance. It should be mentioned, however, that the mouse is probably more sensitive to insulin than humans.

More recently, tissue-specific IR gene disruption was also achieved using the Cre/loxP system. These mutants were used to investigate the pathophysiological consequences of insulin resistance restricted initially to a single target tissue. Surprisingly, muscle-specific IR knockout (MIRKO) mice appeared to have normal glucose homeostasis (Brüning *et al.*, 1998). This was unexpected, as insulin resistance in skeletal muscle is among the earliest detectable defects in NIDDM. However, insulin-stimulated muscle glucose uptake and glycogen synthesis were severely impaired in these mice, whereas insulin-stimulated glucose transport in fat tissue was increased. MIRKO mice presented elevated fat deposits, serum triglyceride and free fatty acid levels, and so they appear to maintain normoglycemia in part by shunting substrates from muscle to fat (Figure 3; Kim *et al.*, 2000). Thus, insulin resistance confined to skeletal muscle can lead to dyslipidemia and obesity, but not to diabetes. In contrast to MIRKO mice, liver-specific IR knockout (LIRKO) mice exhibited severe insulin resistance, glucose intolerance and a failure of insulin to suppress hepatic glucose production (Michael *et al.*, 2000). These mice presented marked hyperinsulinemia due to both a lack of IR-mediated clearance of insulin by the liver and increased insulin secretion due to an almost 6-fold greater β-cell mass. Despite hyperinsulinemia, LIRKO mice displayed post-prandial as well as fasting hyperglycemia, which implies a much greater role for the liver in the control of glucose homeostasis than had been recognized previously. Another idea that has emerged from this model is that insulin resistance in the livers of NIDDM patients might not be merely a consequence of insulin resistance in skeletal muscle and/or fat tissue, as had been assumed previously: it might have other genetic determinants.

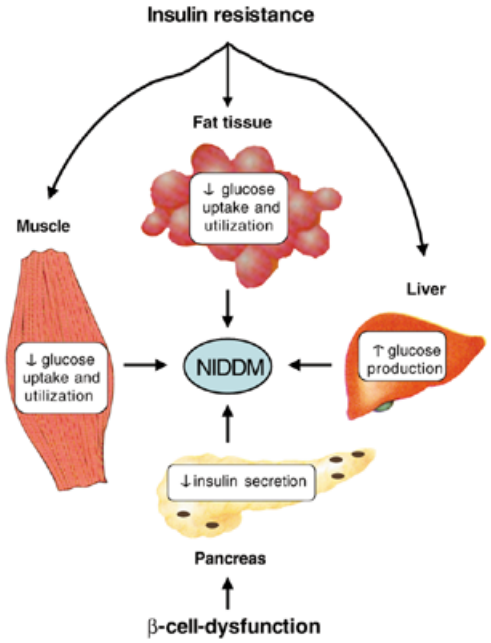

Fig. 2. Insulin resistance in target tissues, along with β-cell failure, leads to NIDDM.

Table I. Mice carrying monogenic defects in some important potential diabetogenes

Global or tissue-specific knockout mice	Striking phenotypic alterations	References
$IR^{-/-}$	Diabetic ketoacidosis. Early postnatal death.	Joshi *et al.* (1996), Accili *et al.* (1996)
MIRKO	Normal glucose homeostasis. Dyslipidemia. Increased adiposity.	Brüning *et al.* (1998), Kim *et al.* (2000)
LIRKO	Severe insulin resistance. Fasting hyperglycemia. β-cell hyperplasia.	Michael *et al.* (2000)
βIRKO	Lack of glucose-induced first phase insulin release. Glucose intolerance with aging.	Kulkarni *et al*. (1999)
$IRS\text{-}1^{-/-}$	Growth retardation. Mild insulin resistance. β-cell hyperplasia. Hyperinsulinemia.	Araki *et al.* (1994), Tamemoto *et al.* (1994)
$IRS\text{-}2^{-/-}$	Severe insulin resistance. Reduced β-cell mass. Early diabetes.	Withers *et al.* (1998), Kubota *et al.* (2000)
$GLUT4^{-/-}$	Moderate insulin resistance. Glucose intolerance. Decreased adipose mass.	Katz *et al*. (1995)
$GLUT4^{+/-}$	Insulin resistance. Diabetes. Hypertension.	Stenbit *et al.* (1997)
MG4KO	Insulin resistance. Glucose intolerance.	Zisman *et al*. (2000)
FG4KO	Insulin resistance in muscle and liver. Glucose intolerance. Hyperinsulinemia.	Abel *et al*. (2001)
$GLUT2^{-/-}$	Glucose intolerance. Impaired insulin secretion. Early death. Diabetes.	Guillam *et al.* (1997)
$GK^{-/-}$ or β-cell $GK^{-/-}$	Diabetic ketoacidosis. Early postnatal death.	Grupe *et al.* (1995), Terauchi *et al.* (1995)
$GK^{+/-}$ or β-cell $GK^{+/-}$	Impaired insulin secretion. Mild diabetes.	Grupe *et al.* (1995), Terauchi *et al.* (1995)
Liver $GK^{-/-}$	Mild hyperglycemia.	Postic *et al.* (1999)

Finally, neurone-specific IR gene disruption revealed that IR in the brain also plays an important role in energy disposal, fuel metabolism and reproduction (Brüning *et al.*, 2000).

The IRS proteins play a pivotal role in linking the IR tyrosine kinase to downstream signaling pathways, and their relative importance in insulin signaling has been assessed by disrupting the corresponding genes. Mice lacking IRS-1 were growth retarded and exhibited only mild insulin resistance without diabetes (Araki *et al.*, 1994; Tamemoto *et al.*, 1994). Interestingly, these mutants presented increased β-cell mass (hyperplasia), resulting in increased insulin secretion (Figure 3). The phenotype of IRS-1 knockout mice strongly supports the idea that overt diabetes does not develop if the β-cells can secrete adequate amounts of insulin to overcome insulin resistance (Kahn, 1998). In contrast, IRS-2-deficient mice presented marked glucose intolerance and became diabetic (Withers *et al.*, 1998; Kubota *et al.*, 2000). The most striking finding was that the lack of IRS-2 prevented adequate β-cell compensation and that the β-cell mass in these mutants was actually lower than in wild-type controls. It was also shown that insulin secretion in response to glucose decreased as hyperglycemia became more severe. This was the first animal model in which the mutation of a single gene caused both the peripheral insulin resistance and the β-cell deficiency seen in NIDDM.

Mouse models with impaired insulin action

Among the biological effects mediated by IR, stimulation of glucose transport in skeletal muscle and adipose tissue is essential for normalizing post-prandial hyperglycemia. GLUT4 is the main insulin-responsive glucose transporter in these tissues, and it is translocated to the plasma membrane in response to insulin. Surprisingly, GLUT4-deficient mice presented only moderate insulin resistance and impaired glucose tolerance and did not develop diabetes as one might have expected (Katz *et al.*, 1995). The lack of GLUT4 did result, however, in growth retardation, reduced fat tissue, cardiac hypertrophy and a shortened lifespan. The alterations in glucose metabolism in $GLUT4^{-/-}$ mice could be improved by transgenic reconstitution of GLUT4 expression in skeletal muscle, but this did not correct adipose tissue abnormalities (Tsao *et al.*, 1997). Unexpectedly, male heterozygous $GLUT4^{+/-}$ mutants showed insulin resistance and developed diabetes (Stenbit *et al.*, 1997). These mice presented hyperinsulinemia, which persisted throughout their lives, suggesting that decreased glucose transport in muscle or fat can lead to diabetes without hepatic insulin resistance or β-cell failure. More recently, it was reported that mice specifically lacking GLUT4 in muscle (MG4KO) developed severe insulin resistance and glucose intolerance. This indicates that glucose uptake by muscle is more critical for the maintenance of glucose homeostasis than is insulin signaling *per se* in this tissue (MIRKO mice; Zisman *et al.*, 2000). Even more intriguing was the phenotype of mice lacking GLUT4 in fat tissue (FG4KO). These mutants had normal growth and fat tissue deposition, although insulin-stimulated glucose uptake in adipocytes was impaired (Abel *et al.*, 2001). Interestingly, in spite of the apparent normality of the fat tissue itself, these mice developed insulin resistance in both muscle and liver, as well as exhibiting glucose intolerance and hyperinsulinemia. The mechanism by which this fat-cell-specific defect alters insulin action at a distance in muscle and liver remains to be elucidated.

Finally, transgenic mice overexpressing phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis, showed unsuppressed hepatic glucose production, despite hyperinsulinemia, and eventually developed diabetes (Valera *et al.*, 1994). This shows that increased glucose production by the liver can contribute significantly to the development of fasting hyperglycemia in NIDDM.

Mouse models with altered insulin secretion

Next to insulin resistance, β-cell dysfunction is the second most important defect in NIDDM. Several mouse models have been generated with the aim of increasing or decreasing circulating

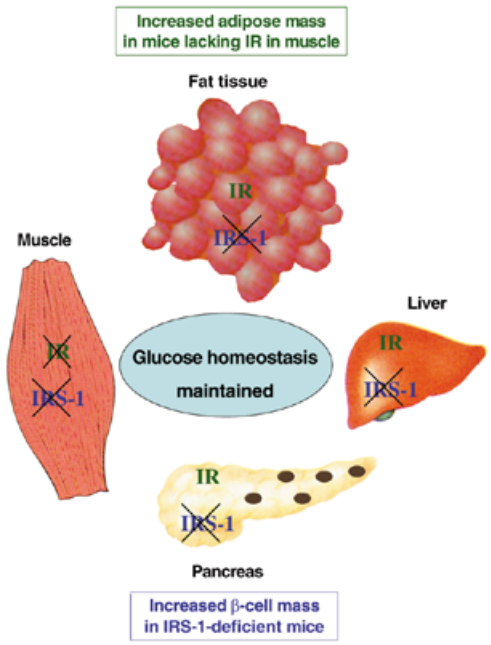

Fig. 3. Glucose homeostasis is maintained in MIRKO and IRS-1$^{-/-}$ mice due to compensatory changes in individual organs. In MIRKO mice, glucose is shunted from muscle to fat. In IRS-1$^{-/-}$ mice, β-cell hyperplasia leads to increased insulin secretion.

insulin levels to mimic this aspect of NIDDM pathogenesis. Two transgenic mouse lines expressing the human insulin gene showed elevated fasting insulin levels (Marban *et al.*, 1989). Although fasting blood glucose levels were normal in these mice, they exhibited glucose intolerance, illustrating that chronic hyperinsulinemia can lead to insulin resistance in the fasting state. The mice have not been tested for IR down-regulation, although this might explain the changed response to insulin. To create a model for insulin deficiency, the two mouse insulin genes *Ins1* and *Ins2* have been disrupted by gene targeting (Duvillié *et al.*, 1997). As one might have expected, pups homozygous null for both genes developed severe diabetic ketoacidosis and died soon after birth. Interestingly, the single gene mutants *Ins1*$^{-/-}$ or *Ins2*$^{-/-}$ were able to maintain normal glucose homeostasis and did not develop insulin deficiency. The total pancreatic insulin content in these mutants reached levels comparable to those found in wild-type controls. This compensation was due partly to 2- to 3-fold greater β-cell mass in these mutants (Leroux *et al.*, 2001).

Glucose-stimulated insulin secretion by the β-cells is a highly regulated process in which the glucose transporter GLUT2 and glucokinase (GK) have been proposed to play important roles. These two proteins are also present in hepatocytes. GLUT2-deficient mice developed hyperglycemia and relative hypoinsulinemia, leading to diabetes and death within the first 3 weeks of birth (Guillam *et al.*, 1997). These mutants presented markedly impaired glucose tolerance, and studies using isolated islets showed that their glucose-stimulated insulin secretion was impaired (Guillam *et al.*, 1997). GLUT2$^{-/-}$ mice could be rescued by transgenic reconstitution of GLUT2 expression in β-cells (Thorens *et al.*, 2000). The role of GK as a glucose sensor in glucose-stimulated insulin secretion was confirmed fully by targeted disruption of the GK gene. Homozygous GK$^{-/-}$ mutants or β-cell-specific GK$^{-/-}$ mice developed severe diabetic keto-acidosis and died soon after birth (Grupe *et al.*, 1995; Terauchi *et al.*, 1995). Transgenic reconstitution of GK expression in β-cells rescued GK$^{-/-}$ mutants and reverted diabetes in 50% of the mice (Grupe *et al.*, 1995). The phenotype of heterozygous GK$^{+/-}$ and β-cell GK$^{+/-}$ mutants, which was characterized by impaired insulin secretion and mild diabetes (Bali *et al.*, 1995; Grupe *et al.*, 1995; Terauchi *et al.*, 1995), was more informative. It is similar to that of maturity onset diabetes of the young type 2 (MODY-2), a monogenic form of NIDDM that results from heterozygous point mutations in the GK gene. On the other hand, mice lacking GK in the liver showed only mild hyperglycemia, indicating that β-cell GK is more crucial than liver GK for maintaining glucose homeostasis (Postic *et al.*, 1999).

ATP-sensitive K^+ channels (K_{ATP} channels) have also been thought to play a role in the regulation of both glucose- and sulfonylurea-induced insulin secretion. The K_{ATP} channels in the β-cell comprise an inward rectifer, Kir6.2, and a sulfonylurea receptor, SUR1. Human patients with mutations in either of the corresponding genes present persitant hyperinsulinemic hypoglycemia of infancy (PHHI). Both Kir6.2$^{-/-}$ and SUR1$^{-/-}$ mice, which lack K_{ATP} channels, exhibited less dramatic phenotypes (Miki *et al.*, 1998; Seghers *et al.*, 2000). Kir6.2$^{-/-}$ mice showed only mild glucose intolerance despite defective insulin secretion, presumably due to insulin hypersensitivity secondary to loss of K_{ATP} channel in skeletal muscle. Interestingly, SUR1$^{-/-}$ mice were both more hyperglycemic when injected with glucose, and more hypoglycemic during fasting, than were controls. Insulin secretion studies with islets from SUR1$^{-/-}$ mice further revealed possible K_{ATP}-independent pathways for the regulation of insulin secretion.

Finally, it has emerged from recent studies that insulin signaling through IR in the β-cell itself could also be important for insulin secretion. Indeed, β-cell-specific IR knockout (βIRKO) mice exhibited a selective decrease in the acute insulin release that normally takes place in response to glucose, and they progressively developed glucose intolerance (Kulkarni *et al.*, 1999), which also occurs in NIDDM patients. However, when pancreatic insulin levels were monitored in hyperinsulinemic IR-deficient mice, it showed a marked, gradual depletion of insulin stores, indicating that active insulin secretion can take place in the absence of IR (Jackerott *et al.*, 2001). Interestingly, compared to βIRKO mice, the double tissue-specific IR knockout βIRKO–MIRKO mice showed improved glucose tolerance, due to improved glucose-stimulated acute insulin release (Mauvais-Jarvis *et al.*, 2000). Muscle and β-cells thus seem to communicate in order to maintain glucose homeostasis.

Polygenic mouse models of NIDDM

Mice carrying monogenic mutations affecting insulin signaling, action or secretion were also used to create mouse models with multi-gene defects. The first polygenic mouse models of NIDDM are presented in Table II. Mutants double heterozygous null for IR and IRS-1 developed severe insulin resistance in muscle and liver, and 40% of these mice became overtly diabetic at the age of 6 months (Brüning *et al.*, 1997). The development of insulin

Table II. Polygenic mouse models of NIDDM

Knockout mice	Phenotypic alterations	References
$IR^{+/-}IRS\text{-}1^{+/-}$	Severe insulin resistance in muscle and liver. β-cell hyperplasia. Diabetes in 50% adult mice.	Brüning *et al.* (1997), Kido *et al.* (2000)
$IR^{+/-}IRS\text{-}2^{+/-}$	Severe insulin resistance in liver. Limited β-cell hyperplasia. Diabetes in adults.	Kido *et al.* (2000)
$IR^{+/-}IRS\text{-}1^{+/-}IRS\text{-}2^{+/-}$	Severe insulin resistance in muscle and liver. Early diabetes. Marked β-cell hyperplasia.	Kido *et al.* (2000)
$IRS\text{-}1^{-/-}GK^{+/-}$	Insulin resistance. β-cell hyperplasia. Diabetes in adult mice.	Terauchi *et al.* (1997)

resistance was accompanied by compensatory β-cell hyperplasia, which led to hyperinsulinemia to overcome insulin resistance. Mice double heterozygous for IR and IRS-2 knockout mutations also developed diabetes, but insulin resistance in this case was restricted to the liver and β-cell hyperplasia was less pronounced (Kido *et al.*, 2000). Triple heterozygous IR, IRS-1 and IRS-2 knockout mice developed early diabetes, with severe insulin resistance in both muscle and liver and markedly increased β-cell mass (Kido *et al.*, 2000). Thus, a combination of 'minor' defects in the insulin signaling cascade can cause insulin resistance synergistically and lead to NIDDM. A polygenic model of NIDDM was also obtained by combining homozygous null mutation for IRS-1 with heterozygous β-cell-specific GK gene disruption (Terauchi *et al.*, 1997). The double knockout mice developed hyperglycemia despite β-cell hyperplasia and became overtly diabetic with age. These last results indicate that even the combination of mild insulin resistance and a minor defect in insulin secretion can lead to NIDDM.

Concluding remarks

It appears that insulin signaling, action or secretion can be impaired by manipulating the expression of the right diabetogenes in the mouse. The phenotypes of such mutants, ranging from mild defects to severe diabetes, have produced novel insights into our understanding of glucose homeostasis and have provided a wealth of information regarding the mechanisms of insulin resistance, β-cell dysfunction and NIDDM pathogenesis. Mice with unexpectedly mild phenotypes exhibited compensatory responses such as activation of alternative metabolic pathways. These studies have also shown that the maintenance of glucose homeostasis involves a dialog between insulin target tissues and pancreatic β-cells. Interestingly, a collapse in glucose homeostasis often results in dyslipidemia, indicating that glucose and fat metabolism are intimately linked. The ability to trigger diabetes by combining gene defects, which in isolation do not lead to major metabolic disorders, has validated the 'diabetogenes' concept of NIDDM. Genetically engineered mice also open the door for investigating the role of gene–gene or gene–environment interactions in the development of NIDDM. In the future, replacement of other specific genes with mutated counterparts should provide new tools for pathophysiological dissection of the disease. Such studies may also identify potential targets for the development of novel therapeutic strategies aimed at improving insulin action or β-cell function in NIDDM.

Acknowledgements

We thank Drs J. Jami and D. Bucchini for their constant, enthusiastic support. We apologize for not being able to discuss many other transgenic studies dealing with several other potential diabetogenes. This work was supported by grants from the Comité de Paris de la Ligue Nationale Contre le Cancer, Ministère de la recherche (ACI) and Université Paris V.

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DOI: 10.1093/embo-reports/kvf078